pyrogenic exotoxin type C (SPE-C) mutant with any amino acid substitutions in the Beta barrel of the B-subunit or a N-terminal alpha helix, whereby the amino acid sequence is altered by any substitution of one or more amino acids. Applicants respectfully traverse this rejection.

With regard to claim 1 and 19, the Examiner contends that the specification is only enabling for specifically named amino acids and does not teach that any amino acid substitution may be changed without causing a detrimental effect to the SPE-C toxin to be produced. The Applicant, however, has provided disclosure in the specification that enables one skilled in the art to readily make mutations that yield a substantially nonlethal SPE-C without undue experimentation.

The specification is replete with information and guidance regarding which amino acid should be substituted therein. Generally speaking, the amino acid to be substituted at a location is selected to include a structural change that can affect biological activity as compared with the amino acid at that location in the wild type SPE-C (pg. 9, ll. 17-20). Such substitutions may be conservative or nonconservative (pg. 9, l. 20). The specification provides a number of specifically exemplified substitutions that result in a structural change that can affect biological activity.

The first such substitution is from one type of charged amino acid to another (pg. 9, l. 22). Specific examples of this type of change can be seen in Table 7, in which lysine at position 135 is changed to aspartic acid, and lysine at position 138 is changed to aspartic acid (pg. 39, table 7). This represents a change from a positively charged amino acid to a negatively charged amino acid.

The second type of substitution that is specified is to change from a charged amino acid to a noncharged amino acid (pg. 9, ll. 22-23). A specific example of this type of substitution is given in Table 7, at page 9. In this example, aspartic acid at position 12 was replaced with alanine (pg. 39, table 7). This represents a change from a negatively charged amino acid to a noncharged amino acid. Table 7 offers another specific example of this type of change in which histidine at position 35 is changed to alanine (pg. 39, table 7). This represents a change from a positively charged amino acid to a noncharged amino acid. Yet another example is provided by changing aspartic acid at position 142 to asparagine (pg. 39, table 7), a change from a negatively charged amino acid to a noncharged amino acid.

The third broad class of substitutions that can cause structural change is substitutions in which cysteine residues are changed, resulting in the disruption of disulfide bonds (pg. 9, l. 23).

The fourth type of substitution specifically stated as a way of making structural changes are those in which substitutions of amino acids result in a change in hydrophobicity. For example, changing tyrosine at position 15 to alanine, or tyrosine at position 17 to alanine (Decl. of Schlievert, p. 5), are examples of changing from hydrophilic amino acids to hydrophobic amino acids. Changing aspartic acid at position 12 to alanine, histidine at position 35 to alanine and tyrosine at position 139 to alanine (pg. 39, table 7) are also illustrative examples of changing from hydrophilic amino acid residues to hydrophobic residues.

The fifth type of substitution that can afford a structural change is a substitution that changes the size of an amino acid residue (pg. 9, l. 25). Examples of this tactic can be seen in a number of specific mutants. For example, by changing tyrosine at position 15 to serine (Decl. of Schlievert, p. 3), tyrosine at position 17 to serine (Decl. of Schlievert, p. 3), tyrosine at position 15 to alanine (Decl. of Schlievert, p. 5), tyrosine at position 17 to alanine (Decl. of Schlievert, p. 5), aspartic acid at position 12 to alanine (pg. 39, table 7), histidine at position 35 to alanine (pg. 39, table 7), lysine at position 135 to aspartic acid (pg. 39, table 7), lysine at position 138 to aspartic acid (pg. 39, table 7), and tyrosine at position 139 to alanine (pg. 39, table 7).

The sixth type of substitution that can be used to cause a structural change is to change to a conformationally restrictive amino acid or analog thereof.

The seventh type of substitution that is specifically stated as one that can cause a structural change that can affect biological activity is to change to a non-naturally occurring amino acid or analog.

Therfore, Applicants assert that the specification does provide more guidance than specific amino acid substitutions.

It is well settled that it is not necessary for a patent applicant to make or test all of the embodiments of their invention to meet the requirements of § 112. In re Angstadt, 190 USPQ 214, 218 (CCPA 1976). The entire mature protein consists of only 235 amino acids in which the specification specifically recites strand 3 of β-barrel 4 of B-subunit 5, loop 6, strand 12, strand 21, α-helix 24, strand 28, loop 29, strand 32, loop 39, loop 43, N-terminal α-helix 51, groove between B subunit 5 and A subunit 61, central α-helix 42, and loop 16 as secondary structural features of this relatively small protein that are suitable locations for mutations yielding a

nonlethal protein (See Figure 1 and specification pages 12 -15). Applicants have successfully made a number of single and double mutants of SPE-C, namely D12A, H35A, N38D, K135 D, K138D, Y139A, D142N, Y17A, N38A, Y17S, N38S, N38A, Y15A/N38A, Y17A/N38A, Y15S/N38S, Y17S/N38S, and disclosed methods sufficient to allow the worker skilled in the art to construct substantially nonlethal SPE-C mutants (specification including at least at pages 28-35). Applicants assert therefore that they have tested a sufficient number of embodiments of their invention, namely D12A, H35A, N38D, K135 D, K138D, Y139A, D142N, Y17A, N38A, Y17S, N38S, N38A, Y15A/N38A, Y17A/N38A, Y15S/N38S, Y17S/N38S, to warrant the breadth of their claim (see specification Example 6 at pages 39-44).

Although Applicants have succeeded in making a number of substantially nonlethal SPE-C mutants, Applicants' invention is enabled even if it requires some experimentation to achieve success for other SPE-C mutants. The necessity of some experimentation does not preclude enablement under § 112; the key is whether the experimentation is undue. In re Angstadt, at 218-219. Even a considerable amount of experimentation is permissible if it is merely routine or if the specification provides reasonable guidance with respect to the direction the experimentation should proceed. Ex parte Jackson, 217 USPQ 804, 807 (Bd. App. 1982).

Applicants provide more than reasonable guidance regarding the direction in which experimentation should succeed. Applicants have specifically identified strand 3 of β-barrel 4 of B-subunit 5, loop 6, strand 12, strand 21, α-helix 24, strand 28, loop 29, strand 32, loop 39, loop 43, N-terminal α-helix 51, groove between B subunit 5 and A subunit 61, central α-helix 42, and loop 16 as secondary structural features of the relatively small protein containing locations suitable for producing nonlethal mutants (see specification pages 12 - 15), identified the amino acids comprising these structural features (see specification pages 12- 13 and Figures 1, 4, 5, and 6), and disclosed guidelines in selecting an amino acid substitution that will result in a structural change that affects biological activity (see specification page 10, lines 10 - 23). Applicants have disclosed methods for obtaining starting material (specification including at pages 34-35), for biologically and biochemically characterizing the starting material (specification including at pages 36-37), for producing SPE-C mutants (specification including at pages 38-39), for analyzing the lethality of SPE-C mutants (specification including at pages 37-38), and for determining the antigenicity of SPE-C mutants (specification including at pages 39-43).

The potential for non-stable SPE-C mutants is within the scope of the claims and does not constitute "undue experimentation," particularly in an art where the level of skill is so high. The Examiner alleges that the specification does not provide guidance on how multiple amino acids can be deleted, substituted, or inserted for the production of a stable protein.

Furthermore, the Applicants respectfully disagree with the Examiner regarding the relevancy of protein stability in this invention. An important requirement for a mutant to function as a vaccine is not stability but nonlethality and antigenicity. The protein does not have to remain intact to serve these functions. When a phagocytic cell engulfs mutant SPE-C toxin, the protein is taken up into an intracellular vesicle. This phagocytic uptake of the mutant toxin is dependent on antigenicity, not stability. Acidification of the vesicle activates proteases to degrade the SPE-C toxin into peptide fragments, typically 11 to 30 residues in length. The vesicle containing the SPE-C peptide fragments fuses with vesicles containing MHC class II where the peptide fragments are bound by MHC class II and transported by MHC class II to the surface of the cell. When helper $CD4^+$ T cells recognize the SPE-C toxin derived peptides bound to MHC class II molecules on the surface of a B cell, they stimulate the B cell to produce and secrete anti-SPE-C antibody. When inflammatory $CD4^+$ T cells recognize the SPE-C toxin derived peptides bound to MHC class II on the surface of a macrophage, they activate the macrophage to destroy any group A streptococci in its intracellular vesicles. Since MHC class II binding of peptide and the subsequent $CD4^+$ T cell activation is not dependent on the stability of the SPE-C toxin, the Examiner's rejection on the basis of protein stability is not relevant to the present claims.

The Examiner supports these assertions regarding protein stability by citing several references dating between 1984 and 1991. The Examiner supports Thomas E. Creighton's 1984 teachings related to hemoglobin and its stability after a single amino acid substitution by citing Nosoh et al. The Examiner, however, fails to recognize that Nosoh et al directly contradicts the Examiner's use of Thomas E. Creighton's 1984 teachings regarding hemoglobin. Nosoh et al notes that the strategy for stabilizing proteins differ from protein to protein and that any generalized mechanisms for protein stability have not yet been presented. Yet the Examiner attempts to characterize the stability of the SPE-C mutants based on prior art relating to hemoglobin, a protein that has significant structural differences when compared to SPE-C. Most significantly, hemoglobin contains 4 heme groups that have an iron atom at their centers.

The Examiner also cites Thomas E. Creighton's 1989 teaching that it is difficult to know which amino acid to change and which is the best residue to substitute for the desired structural and functional effect. The specification, however, provides a roadmap for one skilled in the art to readily construct mutant SPE-C. Applicant's use of crystal structures allowed superpositioning of the SPE-C that teaches the particular structural domains and specific residues of SPE-C that contact MHC class II molecules, T-cell receptors, and liver renal tubular cell receptors (See specification pages 12-15). After identifying the specific secondary structural features and residues, the specification 1) states that there can be more than one amino acid substitution and sets forth a preferred range (specification bridging pages 8 and 9); 2) states that a substitution may be conservative or non-conservative (specification including at page 10, line 13); and 3) provides seven guidelines to selecting an amino acid substitution that results in a structural change that affects biological activity (specification including at page 10, lines 13-23). For example, amino acid substitutions in the N-terminal α-helix need to (1) have hydroxyl groups to interact with the exposed amide nitrogen or (2) be negatively charged to interact with the partial positive charge present at the N-terminus of the α-helix (specification including at page 10, lines 20-23).

Accordingly, Applicants respectfully request the Examiner to withdraw the comments regarding the Creighton and Nosoh et al references and the rejection based on these references. Applicants respectfully submit that the claims are fully enabled by the specification and request withdrawal of this rejection.

Rejection of Claims Under 35 USC § 103 - Goshorn et al. in view of Hartwig et al.

The Examiner rejected claim 1 under 35 USC § 103(a) as obvious over *Goshorn et. al.* in view of *Hartwig et. al.* (International Immunology, 5(8): 869-875 (1993)). Applicants respectfully traverse this rejection.

The combination of *Goshorn et al.* and *Hartwig et al.* only suggests that mutations can be made, that some mutations might affect T lymphocyte activity, and that mutants that affect T lymphocyte activity may be suitable for vaccination purposes. The combination of references, however, fails to disclose or suggest how to make a mutation that is non-lethal.

Goshorn et al. does not disclose or discuss the secondary structure of the mutant SPE-C toxin recited in claim 1 nor does it teach specific amino acid changes in the β-barrel of the B-

subunit or the N-terminal α-helix. Rather, *Goshorn et al.* teaches only the primary structure of the SPE-C toxin by disclosing the amino acid sequence for SPE-C. This reference suggests that it might be desirable to study SPE-C through site-directed mutagenesis; however, the reference fails to mention even a hypothetical mutant of the SPE-C protein or DNA encoding such a mutant. There is no teaching of any particular mutation that would be substantially nonlethal compared with a protein that substantially corresponds to wild type SPE-C toxin.

Hartwig et al. discloses several mutations of SPE-A and the effect of these mutations on T lymphocyte stimulatory activity. There are some similarities between the structures of SPE-A and SPE-C; however, none of the SPE-A mutations reported by *Hartwig et al.* correspond to the residues recited in the present claims regarding SPE-C. *Hartwig et al.* only discloses mutations to SPE-A and does not suggest any mutations to a different protein such as SPE-C. The residues dislcosed by *Hartwig et al.* are approximately 40 or more amino acids away on a different part of a different protein. There is no suggestion that one type of secondary structural feature is preferable for making nonlethal mutations of SPE-C or that nonlethal mutations of SPE-C can be prepared by substituting an amino acid in a β-barrel of a B-subunit or a N-terminal α-helix.

In contrast, the Applicant teaches (1) the particular secondary structural domains and specific residues of SPE-C that contact MHC class II molecules, T-cell receptors, and liver renal tubular cell receptors and (2) that altering amino acids in these particular secondary structural domains produces nonlethal mutations of SPE-C (specification, including at pages 12-15). There is no teaching in *Hartwig et al.* of any particular mutation that would be substantially nonlethal when compared to wild type SPE-C toxin. *Hartwig et al.* does not suggest specific mutations corresponding to aspartic acid-12, tyrosine-15, tyrosine-17, histidine-35, or asparigine-38 in SPE-C. Consequently, *Hartwig et al.* does not enable one skilled in the art to construct substantially nonlethal mutants of SPE-C.

Applicants respectfully submit that the combination of *Goshorn et al.* and *Hartwig et al.* does not render the present invention obvious and respectfully request withdrawal of the objection.

Summary

Claims 1, 3 - 10 and 17 - 19 are in condition for allowance. The Examiner is invited to contact Applicants' undersigned representative at the telephone number listed below if doing so will expedite the prosecution of this patent application.

Respectfully submitted,

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